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CORNELL UNIVERSITY
AGRICULTURAL EXPERIMENT STATION

THE BOTRYTIS BLIGHT OF TULIPS

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ITHACA, NEW YORK
PUBLISHED BY THE UNIVERSITY

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THE BOTRYTIS BLIGHT OF TULIPS

TULIP PLANT AFFECTED WITH BOTRYTIS BLIGHT
One-half natural size

THE BOTRYTIS BLIGHT OF TULIPS[1]

EDWIN F. HOPKINS

THE HOST PLANTS

It is reasonably certain that the Botrytis blight is restricted to plants in the genus Tulipa. This genus includes *Tulipa Gesneriana* L., the Darwin, or late, tulips, *T. suaveolens* Roth., the Duc van Thol tulip, embracing the early and forced varieties, and *T. sylvestris* L., the so-called wild tulip. The members of this genus have been under cultivation for so long that it is difficult to refer them to any natural species although the arrangement just given is commonly accepted (Bailey, 1917:3393-3394).[2]

Numerous references to the occurrence of this disease on other hosts have appeared in the literature. However, most of these statements are based on insufficient evidence, and show that the author had under consideration another Botrytis disease and did not attempt to verify his conclusions by cross inoculations. Ritzema Bos (1903a:20), for instance, says that while the disease affects other bulbs, such as hyacinths, gladioli, and certain iris species, the tulip is by far the most susceptible. Klebahn (1905:15-17) takes exception to this statement since he has found the disease on none of these plants except the tulip; furthermore, his experiments show the hyacinth to be immune. He seems to infer that Ritzema Bos was confusing two different diseases, and that possibly, on these hosts, the disease with which he was dealing was caused by *Sclerotium Tuliparum* Klebahn. However, Klebahn states that practical gardeners have told him that on ground which had borne bulbous begonias and dicentra no tulips came up.

Other notes frequently appear by authors who evidently confuse diseases caused by other species of Botrytis with the one under consideration, which is caused by *Botrytis Tulipae* (Lib.) comb. nov. Halsted (1891:352) gives a good example of this in attributing the disease on onions to this organism.

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[1] Also presented to the Faculty of the Graduate School of Cornell University, March, 1920, as a major thesis in partial fulfillment of the requirements for the degree of doctor of philosophy.

ACKNOWLEDGMENT. The author wishes to express his thanks to Professor H. H. Whetzel, of Cornell University, for his many valuable suggestions and for his helpful advice during the progress of the work.

[2] Dates in parenthesis refer to *Bibliography*, pages 359 to 361.

In order to gain some knowledge of the host range of the parasite concerned, numerous cross-inoculations were made by the writer. These experiments, which are summarized under the heading *Pathogenicity* (page 339), prove that the organism concerned does not attack certain of these hosts under artificial conditions, and therefore it probably would not under natural ones. Klebahn (1904, 1905, 1907) had previously made certain cross-inoculations tending to disprove the existence of a generalized type of parasitism in this pathogene.

The disease is restricted, under normal conditions, to the genus Tulipa; and while similar Botrytis diseases occur on the other hosts mentioned, and even on the tulip itself, they are not identical with this one.

VARIETAL SUSCEPTIBILITY

Varieties of both the early tulips, *Tulipa suaveolens*, and the late tulips, *T. Gesneriana*, are susceptible. Likewise, as already mentioned, the wild tulip, *T. sylvestris*, has been proved susceptible. The writer has collected diseased specimens of many varieties of the first two species and has seen the disease on the wild species.

Klebahn (1905:11), in his experiments, tested five varieties of tulips, presumably early varieties, but finding all susceptible he drew no general conclusion from this result. He thinks it would be desirable to compare the susceptibility of the early and the late species.

The writer found the disease on a large number of varieties of both species, and isolated the causal organism; he also succeeded in artificially infecting both species. He had almost concluded that there was not much difference in susceptibility. But in the spring of 1917, in a garden on the Cornell University campus where there was then a severe outbreak of the disease, one variety of late tulips (Baronne de la Tonnaye), which had certainly been exposed to the infection, showed no evidence of the disease. Up to the present time there has not been an opportunity to test this variety further.

THE DISEASE

NAMES

Various names have been applied to the disease. Ritzema Bos (1903a: 19) incorrectly used the name *kwaden plekken*, a term applied by Dutch bulb growers to soil that will not produce tulips. Later Klebahn (1907:3)

showed that there are various causes of these "bad spots," but that usually they are due to *Sclerotium Tuliparum*. Hence the term *kwaden plekken* may not be applied specifically to this disease.

Ritzema Bos (1903a:19) uses also the word *Umfallen* to designate the disease as it occurs on tulip tops because it often causes the stem to break over. The writer believes, however, that this name is better applied to a physiological disease of tulips described by Sorauer (1903:265). "Tulip mould" is the term used by Massee (1899:158) and also by Halsted (1902:438). Later Jacob (1912) states that it is commonly known as "fire."

The writer would suggest the name *Botrytis blight* for this disease, since it causes a typical blight, and although there is another Botrytis disease of tulips, this is the more important one by far.

HISTORY AND DISTRIBUTION

The Botrytis blight of tulips was probably first described in 1830 by Madame Libert, in connection with an herbarium specimen (Crypt. Ard. No. 36). She evidently observed only the sclerotia. She describes the fungus from the sclerotia as *Sclerotium Tulipae* Libert. From her description it is evident that she had studied the sclerotia of *Botrytis parasitica* Cavara (Saccardo, 1888-89).

The disease was first carefully observed by Cavara (1888), in upper Italy. However, Ritzema Bos (1903a:26) thinks Wakker (1885:22) had the same disease under consideration before Cavara's publication appeared. He called it "tulpenziekte." Ritzema Bos (1903a:25) states that the disease had been known in Holland for more than twenty years, but that he is not certain when it first became seriously destructive there. In 1890 affected tulip bulbs were sent to him from Norway. The work of Ritzema Bos, while in some respects not very accurate, was valuable in that it attracted attention to the importance of the disease. He began his studies in 1896 and published several papers on the subject.

Carruthers (1901:246) notes the occurrence of the disease in Northamptonshire and Cambridge, England, in 1901. It was reported near St. Petersburg in 1911 (Elenkin, 1911). Klebahn (1904:18) mentions its appearance in Hamburg, Germany, in 1902, and in other parts of Germany

later. He has done some of the most important work in clearing up the confusion in regard to the various sclerotial diseases of tulips and their life histories.

The appearance of the disease in America was first recorded by Halsted (1902). He had received in 1901, from a grower at Cape May, New Jersey, a diseased specimen, the bulb of which had originally been obtained from Holland. He stated that the disease had also occurred the previous season (1902:438). Obviously, therefore, it was introduced directly from Holland.

In a letter to Professor H. H. Whetzel, of Cornell University, Professor W. J. Morse, of the Maine Agricultural Experiment Station, mentions some unpublished records of the appearance of the disease at Barrington, Nova Scotia, in 1904, at Cobourg, Ontario, in 1906, and at Cartine, Maine, in 1910. There are also later notes. Professor Whetzel and the writer have received or collected specimens from Amsterdam, Sassenheim, and Aalsmeer, in Holland; from Germany; from Ithaca, McGraw, Garden City, Jamaica, and Brooklyn, in New York State; from Madison, Wisconsin; from Manistee, Michigan; from Washington, D. C.; from Bellingham, Washington; and from Carthage, Missouri. There is no doubt that the disease is widespread throughout the United States and Canada, and it probably will be found wherever tulips are grown.

ECONOMIC IMPORTANCE

There are apparently no exact figures available regarding the amount of damage from the Botrytis blight. Both Ritzema Bos and Klebahn speak of the great loss which this disease occasions to both field growers and florists. In fact, Ritzema Bos (1903:91) was engaged for a time by a growers' association to investigate the disease.

Elenkin (1911) reports that in 1911, near St. Petersburg, Russia, 50 per cent of the tulip crop was destroyed by this and other tulip diseases. A collection and observations made by Whetzel at the New York Botanical Garden in 1916 show the Botrytis blight to have been severe there at that time. Occurrence of the disease is recorded also by Stout (1918:241), and letters and specimens from Dr. David Griffiths indicate that it was very prevalent in the Federal Government's bulb gardens at Bellingham, Washington, in 1917.

In the spring of 1917 the writer observed the disease in an epiphytotic condition. Most of the tulip tops in the ornamental beds in the Cornell University campus were severely attacked. These were late, or Darwin, tulips, and counts made on one variety, Spathulata, showed 100 per cent of the leaves diseased, of which 33 per cent were strongly infected and 67 per cent only slightly. Of the stalks, 98 per cent were diseased, 23 per cent severely and 75 per cent slightly. The bulbs all showed slight infections, but it is uncertain whether these were infections by *Botrytis Tulipae* or by *Penicillium* sp. However, in the variety Spathulata 4.6 per cent of unmistakable Botrytis lesions were found, and in the variety Mrs. Grover Cleveland, 5.2 per cent. The presence of sclerotia in these lesions made identification certain. These were probably lesions from the previous year and they show how small an amount of original inoculum is necessary to produce a severe infection on the tulip tops. Accordingly it is impossible to lay too much emphasis on the selection of clean bulbs. The writer believes that this is the most important disease of the tulip in this country.

SYMPTOMS

On the bulbs

On the brown outer skin, or husk, of the affected bulbs, small black sclerotia may frequently be found, about 1 millimeter in diameter. They appear also on the old, dried, flower stalk of the previous season, which sometimes remains attached to the bulb (fig. 22). The removal of this papery, brown skin often reveals lesions on the outer, white, bulb scale which might otherwise have escaped notice. These lesions vary from deep yellow to brown, are usually circular in outline, and have a definite margin which may be somewhat raised. The central part is ordinarily depressed and may have on its surface small black sclerotia (fig. 23). The lesions are formed sometimes at the apex of the bulb, sometimes at the base, but more often in the region between. Less frequently the sclerotia may appear white, which is due to their immaturity. By removing the outer, fleshy scale and examining its inner side, it will be seen that some of the lesions have penetrated almost to the inner surface. They rarely extend into the scales beneath.

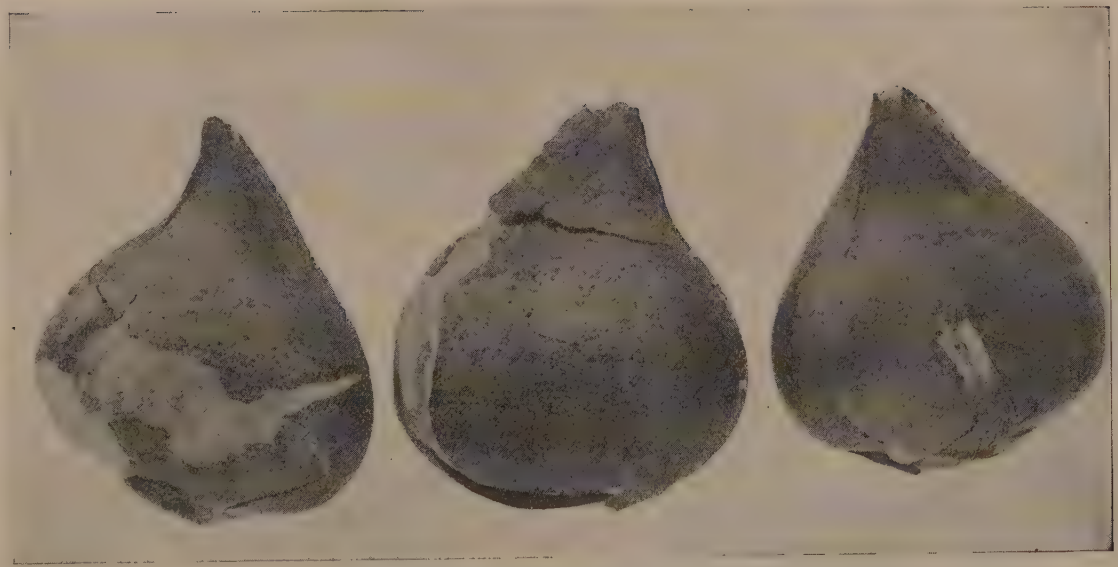

FIG. 22. SCLEROTIA OF BOTRYTIS TULIPAE

Showing them attached to the brown outer skin and the old, dried, flower stalk of dormant bulbs. Natural size

Under warm, humid conditions, a large part of the outer scale may become affected, and in some cases it is so densely covered with sclerotia that these coalesce and form a crust.

Care must be exercised not to confuse the lesions on the bulbs due to the very common Penicillium rot with those caused by *Botrytis Tulipae*.

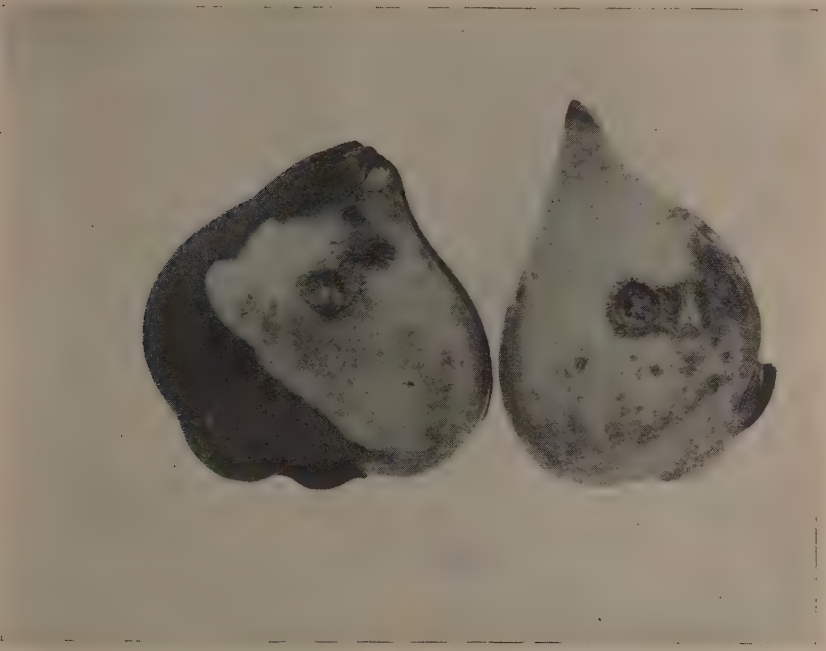

FIG. 23. BOTRYTIS BLIGHT LESIONS ON THE BULBS

The lesions in the outer, fleshy, bulb scale are depressed and show sclerotia on their surface. Natural size

This is especially true of the incipient lesions which, in these two diseases, are often found almost impossible to differentiate. In general, the lesions of the Penicillium rot are more indefinite in outline and of a lighter yellow color, are usually raised and uneven, and do not, of course, have sclerotia on their surface. The appearance of green mold, which forms under favorable conditions, is a distinguishing characteristic.

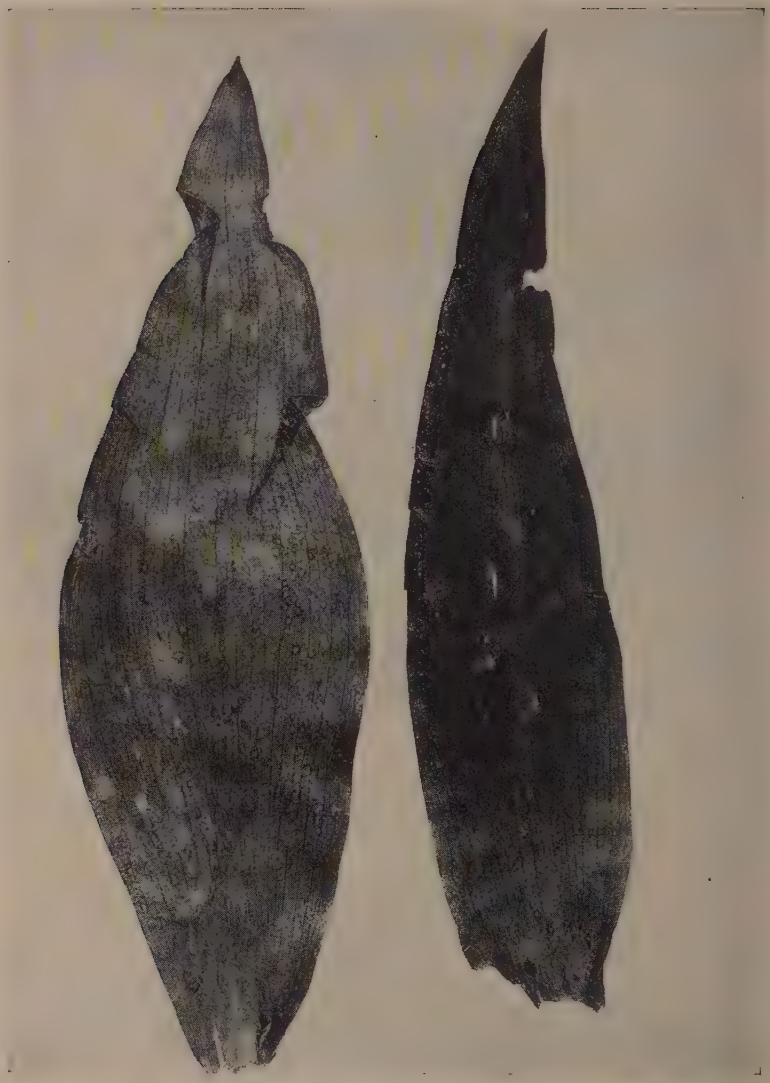

FIG. 24. BOTRYTIS BLIGHT LESIONS ON THE LEAVES
Young lesions. Three-fifths natural size

On the leaves

On the leaves the lesions show first as minute, yellowish spots, somewhat elongate in the direction of the leaf veins and surrounded by a darker,

FIG. 25. BOTRYTIS BLIGHT LESIONS ON THE LEAVES
Lesions of a more advanced stage of development. One-half natural size

water-soaked area. They are slightly sunken and give the leaf a speckled appearance. As they enlarge, the areas become more depressed, the

FIG. 26. BOTRYTIS BLIGHT LESIONS ON THE LEAVES
Peculiar twisting of leaves due to a marginal lesion. Natural size

color changes to a whitish gray with a brownish tinge, and a translucent or water-soaked area appears about the margin. At this stage the margins of the lesions are quite definite (fig. 24). Toward the center abundant conidiophores are often produced. Under favorable conditions the lesions enlarge still farther, coalesce, and frequently involve the entire leaf. If a lesion develops toward the base of a leaf, it may cause the leaf to break over. When an infection takes place on the margin of the leaf near the tip, there results the characteristic appearance shown in figure 26. This wrinkling and bending of the leaf to one side is due to the more rapid growth of the healthy tissue opposite the lesion. (Klebahn, 1905:4).

Both young and old lesions are found on the same leaf showing that infections take place continuously (fig. 25). The outer sheathing leaf is likely to be attacked before the others, probably being infected as it emerges from the bulb. It bends downward and usually is abundantly covered with conidiophores (fig. 27).

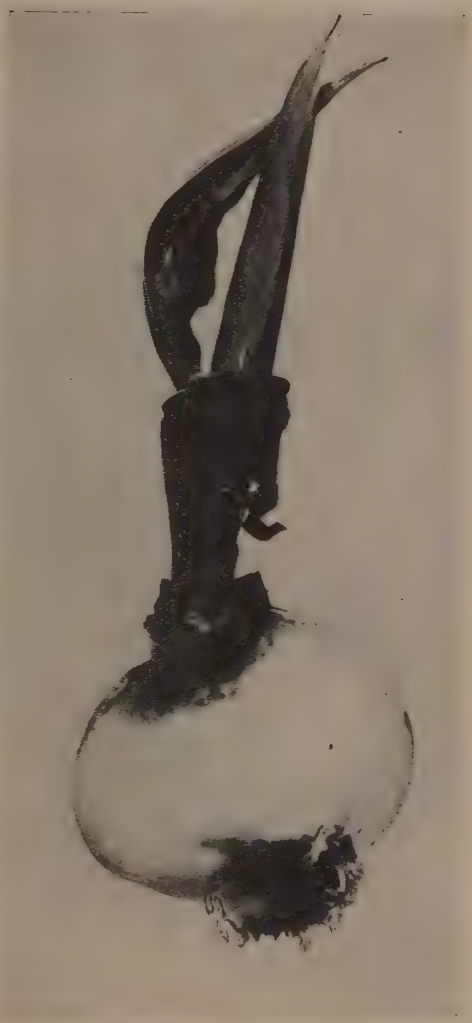

FIG. 27. A DISEASED PLANT

Showing the outer sheathing leaf infected by contact with a lesion at the tip of the bulb. Note the abundance of conidiophores which give rise to inoculum for secondary infections. Natural size

On the flowers

The lesions on the flowers (Plate XXXIII) are very striking, especially on red varieties of tulips. They begin as minute spots, whitish to light brown, the color being bleached from the perianth. These spots are

FIG. 28. LESIONS ON THE FLOWER
Natural size

evenly distributed over the surface and usually show no Botrytis fructification (fig. 28). After the lesions enlarge, however, they turn a deeper brown and involve the entire segment of the perianth, which finally becomes dry and wrinkled. At this time they show abundant conidiophores. The whole flower may be affected and appear blighted.

FIG. 29. BUD BLIGHT SHOWING THE BLANCHING OF THE INFECTED TISSUE
Natural size

Indeed, this blighting may take place when the flower is still in the bud and prevent it from opening. Such a typical bud blight is reproduced in figure 29.

On the stalks

While the lesions on the stalks are still small, they have much the same appearance as those on the leaves except that they are more elongate and more depressed. They are of a light brownish color in the center, and are surrounded by a water-soaked area. Older lesions near the base of the stalk appear as dark brown patches and often bear sclerotia on their surfaces, while those higher on the stalk, usually originating in the leaf axils, are grayish white and are covered with conidiophores. Both this blanching effect and the conidial layer are well illustrated in figure 30.

The extension of the lesion through the stem causes the latter to weaken at the point of attack and break over. If the lesion is near the base, the whole plant topples over; if it is higher up, the flower droops.

ETIOLOGY

Nomenclature

The tulip disease under discussion is caused by *Botrytis Tulipae* (Libert) comb. nov. A sclerotial form, referred to the form genus Sclerotium by Madame Libert, in 1830, belongs to this species. She called it *Sclerotium Tulipae* and was the first to describe it (Crypt. Ard., no. 36). As previously mentioned, she apparently did not observe the conidial form. Her original description, which the writer has not been able to see, is taken from Klebahn (1907:5) who quotes it as follows:

> *Sclerotium Tulipae*, N. Sparsum, adnatum, parvum, ovale, pallide fuscum, laeve, demum nigrum, rugosum, intus album. Ad caules, pericarpia et semina *Tulipae Gesnerianae*. Autumno.

In 1836 the species was again designated as *Sclerotium Tulipae* by Weinmann (1836:647). Sometime between 1841 and 1859 it was once more described in connection with an herbarium specimen, this time by Westendorp. He called it *Sclerotium entogenum* (Herb. crypt. Belg., no. 827). Finally Cavara (1888) described the fungus more completely,

FIG. 30. DISEASED STALKS
Natural size

including both the conidial and the sclerotial stages, which he assumed belonged to the same species. His description is as follows:

Botrytis parasitica nov. sp. Hyphis cinereis sparsis, erectis, articulo basali inflato; gonidiis ovatis, magnis, breviter pedicellatis, in ramulis minutis, capitatis, umbellatim dispositis; hyalinis vel dilute cinereis, 16–20 x 10–13 μ.

Forma scleroziale.

Sclerotium Tulipae Lib. Haemisphaericum, vel oblongum, nigrum, vix rugosum, superficiale vel immersum, intus albidum ½–1 mm. latum.

Hab. Ad folia, caules, petala, et capsulas *Tulipae Gesnerianae* in Horto botanico ticinensi.

Cavara (1888:432) justifies his description of this species as a new one on the basis of morphological differences and also because of its strong parasitic action. In his discussion of nomenclature, he says that *Sclerotium Tulipae* Terry, which infects tulips in the south of France, according to Saccardo (1888–89,) is probably a synonym of *Sclerotium cepivorum* var. *Tulipae* Desm., and perhaps is the same as *Scl. cepae* Desm. Cavara was not able to compare specimens of *Scl. Tulipae* Terry with *Scl. Tulipae* Lib. The writer, however, had an opportunity to examine a specimen of the former at the herbarium of the New York Botanical Garden. This specimen consists of three or four sclerotia with no adhering plant material. The sclerotia are large, however, and are not those of *Botrytis Tulipae* (Lib.) comb. nov. At the same place the author was able to see some of the collection of Cavara designated *Botrytis parasitica* Cav., and found it to be identical with his own collections.

Massee (1899:383) describes the organism as *Sclerotinia parasitica*. This description was not based on a perfect stage and in Massee's key is placed under " Conodial form only known." Since there is no evidence that the species under consideration is a Sclerotinia, this name is not valid. Massee's description is as follows:

Sclerotinia parasitica, Massee; *Botrytis parasitica*, Cavara. Conidiophores grey, scattered, erect, basal joint inflated; conidia obovate, large, shortly pedicellate, on short umbellately arranged branchlets, hyaline or tinged grey, 16–21 x 10–13 μ; sclerotia formed in the parenchyma of the host, globose-depressed, smooth, greyish, then black, 2–3 mm. diam., sometimes numerous, and forming black crusts.

Botrytis on leaves, stem, and flowers of cultivated tulips; sclerotia more especially on the bulbs.

Distr. Holland, Britain.

A consideration of these facts has led to the following designation of the species:

Botrytis Tulipae (Libert) comb. nov.

Sclerotium Tulipae Lib. Crypt. Ard., no. 36. 1830.
Sclerotium Tulipae Weinn. Hym. Ross., p. 647. 1836.
Sclerotium entogenum West. Herb. crypt. Belg., no. 827. 1841–1859.
Botrytis parasitica Cav. Appunti die Pathologia Vegetale. Ist. Bot. R. Univ. Pavia. Atti 2:1:432. 1888.
Sclerotinia parasitica Massee. A text book of plant diseases, p. 383. 1899.

Botrytis Tulipae (Libert) comb. nov. may be briefly described as follows: Mycelium variable in diameter, often anastomosing, branches not constricted at the base; conidiophores arising directly from the mycelium, erect, brown in color, proliferating, twisting on their axils when dry, slightly swollen at the base; branches of conidiophore arising at an angle of about 60 degrees, dichotomous, not streptiform, apices swollen; conidia large, 12-24 x 10-20 μ, obovate, reddish brown in mass; microscopically gray to hyaline, smooth with a short stalk, often or commonly not remaining attached; sclerotia at first white, finally black, small, 1-2 millimeters in diameter, circular or somewhat elliptical in outline, flattened vertically and often convex. Microconidia globose, about 3 μ in diameter, occurring on special penicillate, obclavate conidiophores arising in white tufts from the substratum (fig. 40). Parasitic on *Tulipa* spp.

Cavara (1888:432) says that *Sclerotium entogenum* West. develops on the stalks of asparagus and does not differ greatly from *Scl. Tulipae* Lib.; he says, moreover, that Westendorp thinks *Sclerotium entogenum* West. should be regarded as the type of the species. *Sclerotium Tuliparum* Klebahn is a species which must not be confused with *Botrytis Tulipae.* It is a large, sclerotial form with which no conidial stage has yet been connected.

The species must, therefore, still be classified among the Hyphomycetes of the Fungi Imperfecti. It is placed there by Lindau (1900:435) in the subgroup Mucedinaceae-Hyalosporae-Botrytideae.

Klebahn (1904:21), from his studies on *B. Tulipae*, seems certain of the connection of the sclerotia occurring on the tulip bulbs with the conidial form on the leaves. However, as he did not use pure-culture methods, it seemed desirable to clear up this point. Pure cultures were made by the writer from the sclerotia occurring on the bulb and from conidia on the leaves of the same tulip plant. These cultures were identical, and when inoculated into sterilized tulip leaves both produced normal conidia and sclerotia. Both also caused infection of healthy tulip plants.

Morphology

The mycelium shows no peculiar characteristics. It varies in diameter, depending on the conditions under which it lives. The branches are not constricted where they join the parent hypha. Anastomosing is frequent (fig. 31). The conidiophores, when mature, are deep brown in color except near the base, where they are hyaline. They are indeterminate in length, for under favorable conditions, after forming one head of conidia terminally, the main axis may proliferate and form another head (fig. 50). This may be repeated until finally there are several clusters of conidia on a single conidiophore (fig. 32). It should be remembered that although some of these clusters appear to be lateral, they are really formed terminally. When

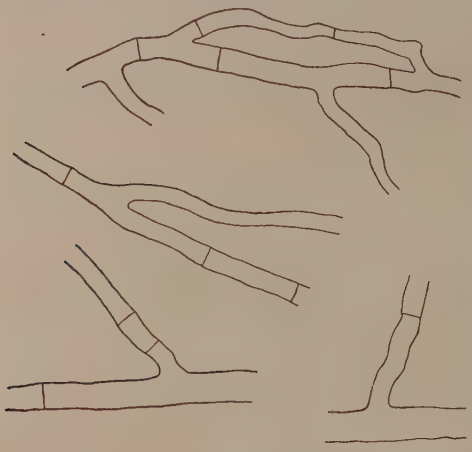

FIG. 31. MYCELIUM OF BOTRYTIS TULIPAE. × 600
Type of branching and anastomosing of the hyphae. (Camera-lucida drawing)

FIG. 32. CONIDIOPHORE OF BOTRYTIS TULIPAE. × 67
A conidiophore unmounted. The several clusters of conidia due to proliferation, and the twisting of the stalk when dry, are both visible. (Camera-lucida drawing)

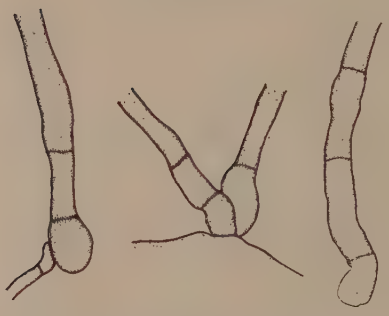

FIG. 33. SWOLLEN BASES OF CONIDIOPHORES OF BOTRYTIS TULIPAE. × 600
(Camera-lucida drawing)

dry, the conidiophore is flattened and twisted on its axis, and is slightly swollen at the base (fig. 33).

The branches of the conidiophore rise from the main stalk at an angle of about 60 degrees, and their ultimate ends, which bear the conidia, are somewhat swollen. The conidia are produced on these swollen ends by a pushing out of the protoplasm in a bud-like manner, but they shortly assume a definite shape. When mature, they remain attached by short sterigmata (figs. 34 and 35). Various stages of conidial formation

FIG. 34. ATTACHMENT OF CONIDIA OF BOTRYTIS TULIPAE. × 600

Camera-lucida outline of conidia shown in fig. 35. Proliferation of the conidiophore has taken place after the formation of conidia on the head.

FIG. 35. ATTACHMENT OF CONIDIA OF BOTRYTIS TULIPAE. × 840

Note the short sterigma. These conidia are mature. (Photomicrograph)

are shown in figures 36 and 37.[3] Usually young conidiophores were chosen for study because of the firmer attachment of the conidia. The preparation of the mounts required considerable patience because of the delicate attachment of the conidia, and oftentimes many mounts were prepared before one was obtained which showed clearly the details of structure. When young, the conidia are hyaline, but as they mature they assume, in mass, a brownish color. Microscopical examination shows that most of this color is in the spore wall. The conidia are obovate, and when shed, the short sterigmata may sometimes be seen still attached to the spores.

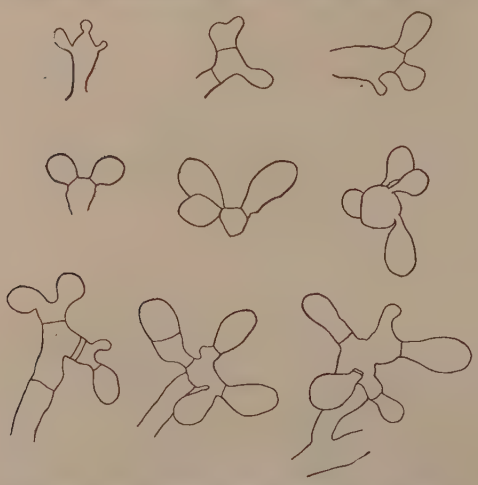

FIG. 36. DEVELOPMENT OF CONIDIA OF BOTRYTIS TULIPAE. × 600

Illustrating the bud-like manner of their formation. (Camera-lucida drawing)

The conidia vary considerably in length and to a less extent in width, but this variation does not depart greatly from a mean which is more or less constant. This is shown graphically in figure 38, which represents the measurements of one hundred spores. One curve expresses the variation of the spore length and the other that of the spore width.

The spores measured were from single-spore cultures and developed on sterilized tulip leaves in petri dishes. Abundant conidial fructifications were formed which were practically identical in appearance with those occurring in nature. They were mounted in the mounting fluid previously described[3] and were measured under the oil-immersion lens by

[3] In studying the detail of the conidiophore, especially the attachment of the conidia, satisfactory mounts were obtained by first adding to the material on the slide a drop of 70-per-cent alcohol in order to "wet" the conidiophores rapidly. The material was then flooded immediately with a mounting fluid prepared by mixing equal parts of 2-per-cent potassium acetate in water and 40-per-cent glycerin in alcohol and then adding a trace of copper acetate. The excess mounting fluid was removed with filter paper and the mount covered with a cover glass. Such mounts keep very well, do not dry out, and may be kept permanently when ringed with balsam or gold size.

means of camera-lucida drawings. One hundred spores were outlined and the outlines measured with a millimeter rule. The error in measuring was calculated to be less than 2 per cent. On the basis of these measurements the average limits of variation may be placed at 12-24 x 10-20 μ.

These figures do not show the distribution of the spores within these limits. From figure 38, however, it is apparent that the greater number have a length of 16-17 μ and a width of 9-10 μ. These measurements were checked with those of spores from another culture derived from a different locality. Measurements made of the spores of a large number of Botrytis specimens seem to show that one hundred conidia suffice to establish the mode for a given species.

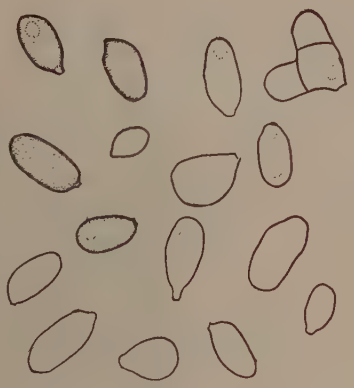

FIG. 37. DEVELOPMENT OF CONIDIA OF BOTRYTIS TULIPAE. × 600

Mature conidia. (Camera-lucida drawing)

The sclerotia as formed in a petri-dish culture, (fig. 39) are at first white, and later, a shiny black. They are circular, elliptical, or somewhat irregular in outline, flattened vertically, and often slightly convex. They might be described by the term "loaf-shaped." Ordinarily the sclerotia are about one millimeter in diameter. Their size may be considerably affected by the amount of drying to which they are subjected.

Physiology

Growth

Botrytis Tulipae grows very readily on the various kinds of media, both liquid and solid, on which it has been planted. In the writer's experimental work the commonest medium employed was potato-dextrose agar. On this the fungus makes a rapid, fluffy mycelial growth, which later becomes appressed to the surface of the agar and the sclerotia then begin to form. These are very numerous and are imbedded in a tough, mycelial membrane which covers the surface of the substratum. As mentioned under *Conidia production*, conidia are rarely formed in such cultures. Moreover, there is scarcely any color production in this medium.

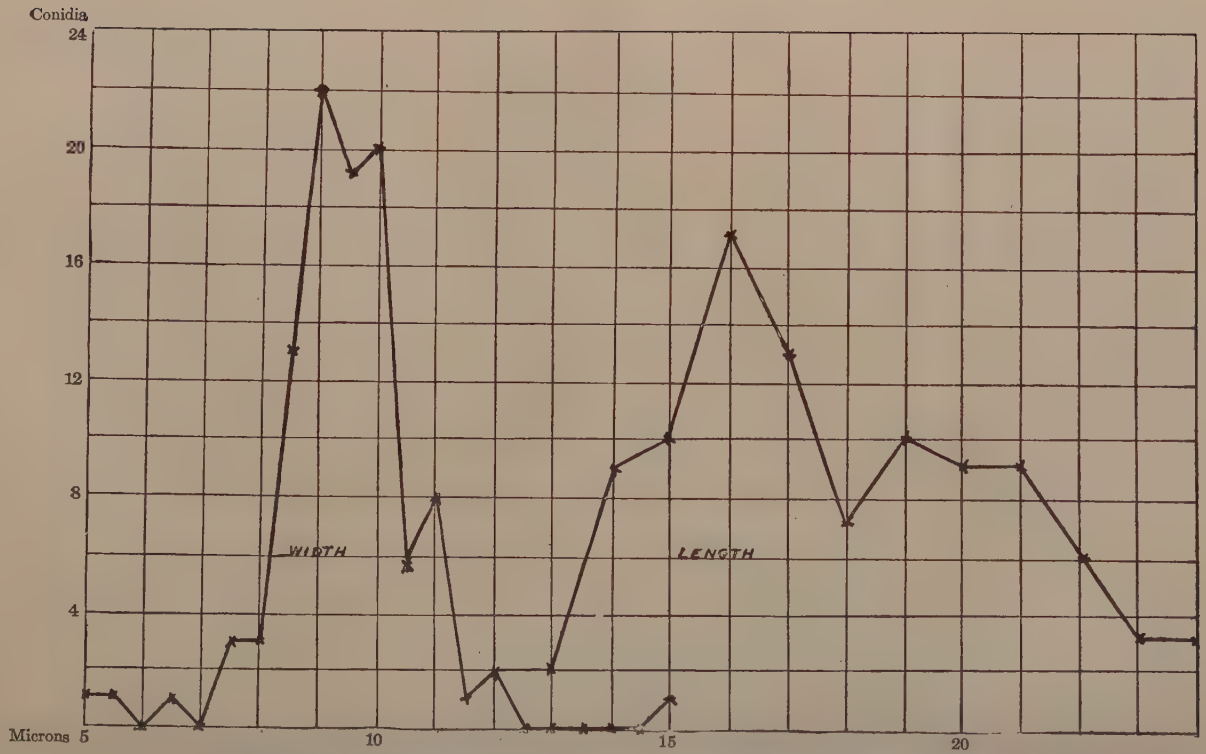

FIG. 38. GRAPHIC REPRESENTATION OF CONIDIAL MEASUREMENTS OF BOTRYTIS TULIPAE

Showing the distribution of 100 conidia. The ordinates show the number of conidia; the abcissae, their length or width in microns

It is of little importance to describe at length the growth on other media: sucrose is utilized as a source of carbon apparently as successfully as glucose; on a glucose solution, without mineral nutrition, the growth is very poor: on plain agar and water the growth is sparse and the mycelium tends to spread; but on agar containing a mineral-nutrient solution, with

FIG. 39. SCLEROTIA OF BOTRYTIS TULIPAE
As formed in a petri-dish culture on potato agar. Natural size

no source of carbon, an excellent growth takes place, even better, perhaps, than that on potato-dextrose agar. This indicates that the carbohydrates of the agar itself are being utilized. With oxalic acid as a carbon source, the growth is poor.

Growth is best on an acid medium, and, although no experiments have been made to determine the range of acidity, good development has

always been observed when the acidity was approximately +20 according to Fuller's scale. Perhaps the range is as wide as that determined by Munn (1917:407-408) for the growth of *Botrytis Allii*.

Conidia production

Although conidia are produced abundantly in nature on tulip leaves, in agar culture under the ordinary laboratory conditions they rarely appear. The fungus tends rather to form sclerotia. However, early in 1917 the writer observed for the first time that conidia had formed under these conditions when some conidiophores developed in five petri-dish cultures. It should be noted that these conidia were produced without any special treatment, and that, although they were produced once or twice afterward, this is rather unusual under the conditions obtaining in ordinary culture vessels.

On March 17, 1917, sterilized tulip leaves in large test tubes, 20 x 2.5 centimeters in size were inoculated with cultures of three different strains in duplicate. In two of these strains a fluffy mycelium soon developed, which spread along the leaves. After about ten days conidiophores began to develop, usually toward the top of the culture, and by March 31 there was an abundant development of conidiophores close to the surface of the leaves just beyond the aerial mycelium as well as on the mycelium itself. The conidia produced were able to cause infection in healthy tulips. This experiment was repeated on April 27, 1917, and the cultures were observed daily in order to note the first appearance of the conidia. This occurred in five cultures on May 8.

Some time later, Professor Whetzel suggested that a partial drying of the plate cultures, after a good growth of mycelium had started, might produce conidia. This experiment was accordingly performed, and the partial drying was accomplished by so placing the petri-dish cover as to expose part of the agar surface, thus permitting more rapid evaporation. The petri dishes were usually left overnight in a dust-free chamber and were tightly covered again the next morning. Conidia were in this way produced successfully in a large number of instances, not only from *B. Tulipae*, but also from other Botrytis species which do not readily fruit in culture. On halves of sterilized tulip bulbs in petri dishes the fungus was also found to fruit abundantly. There seems to be a relation between conidial

production and the relative humidity over the culture, or, to state it more concisely, a relation between the rate of evaporation and conidial production.

There is, perhaps, something inherent in the nature of the host tissue which makes it an especially good substratum for the production of conidia; but it is more likely that the fungus finds in such cultures more variation in the moisture relations. This would explain why, in the experiments with sterilized tulip leaves, the conidia were not produced uniformly throughout the culture but only on a limited area toward the top of the culture tube, where the moisture relations were presumably most favorable.

Dissemination of conidia

The peculiar twisting of the conidiophores, already mentioned, is thought to have some relation to the dissemination of the conidia. In the first place, the conidia fall away very readily. This becomes obvious when an attempt is made to mount conidiophores bearing conidia in a liquid medium or if a conidiophore is jarred slightly. Secondly, the conidiophores are very hygroscopic, and a small change in the atmospheric moisture will cause them to twist with considerable violence and frequently even to dislodge the conidia. This phenomenon can be easily observed in this and in other Botrytis species by placing the specimen under a binocular microscope. Merely breathing on it gently suffices to produce these contortions. It may be concluded that in nature, owing to the frequent changes in humidity that occur, conidia are commonly dislodged in this manner and carried off by air currents.

The conidia are usually wind-borne. The writer has found that inoculations with conidia are best made by either blowing or dusting them on the host plants and subsequently spraying water on them with an atomizer.

Pathogenicity

The pathogenicity of *Botrytis Tulipae* was first demonstrated by Klebahn (1905:6), who inoculated tulip bulbs with sclerotia from a pure culture. However, he appears not to have done much exact inoculation work, and, with the exception of the experiment cited, did not use pure cultures in his infection tests. The writer does not consider that Ritzema Bos

(1903 a : 24) demonstrated the pathogenicity of the organism by his investigations, for he did not use pure-culture methods, but merely developed Botrytis conidia on leaves in moist chambers and then dusted the conidia on sliced bulbs, causing the bulbs thus treated to decay.

Experimental methods

The writer made isolations of the fungus from material obtained from many localities in Holland, Germany, England, Canada, and the United States. These isolations were made both from dry specimens (for it was found that the fungus would retain its vitality for a long time without moisture) and from recently infected plants. Moreover, they were made from sclerotia, mycelium, and conidia from various parts of the host — bulbs, stalks, leaves, buds, perianth, and stamens.

Although Klebahn (1905 : 12) found it difficult to obtain pure cultures from the sclerotia, the writer has experienced little difficulty in isolating from sclerotia by the following method: The sclerotium is rubbed free from all adhering material with a clean piece of cheesecloth, and is dipped for instant in 95-per-cent alcohol to remove the surface film of air, that is, to wet the surface. It is then placed in a 1 : 1000 mercuric-chloride solution for about thirty seconds, after which it is quickly removed with sterilized forceps and placed in a drop of sterilized water in a sterilized petri dish. To thoroughly remove the mercuric chloride, the sclerotium is then rinsed in several successive drops of sterilized water in the same petri dish. Usually six washings are sufficient. The sclerotium, thus prepared, is cut into four pieces and planted on a poured plate of potato-dextrose agar. The whole operation should not take more than five minutes. The writer has used this method in isolating several hundred Botrytis specimens as well as specimens of other fungi, and rarely has a contamination occurred.

Fungi may be isolated from leaf tissue in this way if care is taken not to leave the material too long in the alcohol. In these experiments the mycelium was usually isolated from the leaf tissue and the stems by cleaning the epidermis with alcohol and then peeling it back, or often, when using stems and bulbs, by breaking or splitting them open so as to expose an uncontaminated surface. Small parts of the diseased tissue were then picked out with a sterilized, sharp-pointed scalpel and planted in agar.

Conidia were isolated in several ways. Sometimes they were blown over the surface of the agar from the bent end of a platinum needle. The needle was attached to a piece of glass tubing which served as a blowpipe. The tendency at first was to gather too many spores on the needle, but with practice a sparse sowing was readily made and transfers were then obtained from the resulting colonies. Another method was to pick off, with sharp-pointed forceps, a single conidiophore, under a binocular microscope if possible, and then touch it to an agar plate in several places. Pure cultures usually resulted from some of these plantings, and often all the cultures were pure.

Pure line cultures were obtained in two ways: first, by planting the fungus on a poured plate of plain agar and water, which caused the mycelium to spread out in its growth so that a single mycelial tip could be marked under the low power, cut off, and transferred; secondly, by the isolation of a single spore. In the latter method, which was the one most frequently employed, care was necessary lest more than a single spore should be obtained. A thin layer of agar containing a few conidia was poured into a petri dish and the spores were allowed to germinate slightly. After a conidium was marked and transferred to a poured plate, a microscopical examination was always made to ascertain positively that not more than one spore had been cut out. The growth of these cultures on potato agar is characteristic and is described under *Physiology* (page 355).

Both mycelium and conidia were used as inoculum. The mycelium inoculum was prepared by growing the fungus in a petri-dish culture until the colony had reached the size of an inch or so in diameter. Small cubes of agar containing mycelium were then cut with a sterile scalpel from the edge of the colony and placed on the plant part to be inoculated, with the side containing the mycelium against the host. To prevent the inoculum from drying out, the plants were either placed in a large, moist chamber or covered with a bell glass or a lamp chimney. When it was desired to injure the inoculated parts, this was done by pricking a sterile, sharp-pointed scalpel through the agar block into the host tissue.

In using conidia, difficulty was at first experienced in attempting to spray the plants with spore suspensions in water. No infections resulted. As already mentioned, this is explained by the fact that the conidia are

not readily wet with water and consequently the water sprayed contained but few conidia in suspension. This was demonstrated by a microscopical examination of the drops on a slide sprayed with the suspension. Very few spores were found, and hence the chances for infection were slight. Since this method was unsatisfactory, an attempt was made to secure infection by dusting the conidia on with a camel's-hair brush. This proved to be very successful, and abundant infection resulted, both on dormant bulbs and on growing tulip plants. Plants inoculated in this manner were sprayed with sterile water from an atomizer and kept moist overnight.

Results of inoculations

The results obtained from inoculating dormant tulip bulbs with mycelium are shown in table 1. From this table it is clear that the dormant bulbs must be injured at the point of inoculation in order that

TABLE 1. RESULTS FROM INOCULATING DORMANT TULIP BULBS WITH MYCELIUM

Culture	Number of bulbs inoculated		Number of bulbs infected	
	Injured	Uninjured	Injured	Uninjured
B. 298	4	2	4	0
B. 163	2	1	2	0
B. 112	2	1	2	0
B. 149	2	1	2	0
B. XVII	2	1	2	0
B. XXVII	2	1	2	0
B. XXXII	2	1	2	0
Total	16	8	16	0

* The arabic numerals represent cultures from domestic sources, and the roman numerals, those of foreign origin.

mycelium may infect them. Briefly stated, the entire sixteen of the dormant bulbs which had been injured were infected by the inoculation, whereas none of the eight uninjured bulbs were thus affected. On the other hand, when ten bulbs were inoculated just after the flowers had been cut, all became infected, although only five were previously injured.

A summary of the results obtained from inoculating tulip stems with mycelium appears in table 2. From this table it is evident that the tulip stems inoculated with twelve different cultures of *Botrytis Tulipae*, five

TABLE 2. RESULTS FROM INOCULATING TULIP STEMS WITH MYCELIUM

Culture*	Number of stems inoculated		Number of stems infected	
	Injured	Uninjured	Injured	Uninjured
B. 298	3	3	3	3
B. XXVII	4	5	4	3
B. XXV	4	5	2	2
B. XXVI	4	5	4	5
B. XVII	4	5	4	5
B. XXXII	4	5	4	5
B. 112	3	3	3	2
B. 143	3	3	3	3
B. 149	3	3	3	3
B. 150	3	3	3	3
B. 163	4	5	4	5
B. 414	1	2	1	1
Total	40	47	38	40

* The arabic numerals represent cultures from domestic sources, and the roman numerals, those of foreign origin.

TABLE 3. RESULTS FROM INOCULATING TULIP LEAVES WITH MYCELIUM

Culture*	Number of leaves inoculated		Number of leaves infected	
	Injured	Uninjured	Injured	Uninjured
B. XXVII	1	2	1	0
B. XXV	1	2	1	2
B. XXVI	1	2	1	2
B. XVII	1	2	1	2
B. XXXII	1	2	1	2
B. 163	1	2	1	2
B. 414	1	2	1	1
Total	7	14	7	11

* The arabic numerals represent cultures from domestic sources, and the roman numerals, those of foreign origin.

from foreign sources and seven from domestic, showed thirty-eight infections out of forty when pricked with a needle after inoculation, and forty out of forty-seven when not injured in this way.

A summary of the results obtained from inoculating tulip leaves with mycelium is given in table 3. All seven leaves showed infections when inoculated and injured; of the uninjured leaves, eleven out of fourteen showed infections.

Tulip flowers were inoculated with mycelium, with results as given in table 4. The tulip flowers, like the leaves, were all seven infected when an injury was made at the point of inoculation. Out of fourteen not so injured after inoculation, twelve showed infections.

TABLE 4. RESULTS FROM INOCULATING TULIP FLOWERS WITH MYCELIUM

Culture*	Number of flowers inoculated		Number of flowers infected	
	Injured	Uninjured	Injured	Uninjured
B. XXVII	1	2	1	1
B. XXV	1	2	1	2
B. XXVI	1	2	1	2
B. XVII	1	2	1	2
B. XXXII	1	2	1	2
B. 163	1	2	1	2
B. 414	1	2	1	1
Total	7	14	7	12

* The arabic numerals represent cultures from domestic sources, and the roman numerals, those of foreign origin.

It should not be deduced that the lower proportion of infections in the uninjured leaves, stems, and flowers was owing to the inability of the fungus to penetrate uninjured tissue. The writer attributes it rather to experimental error; for the inoculum was more likely to be lost or dried out before infection had opportunity to take place than when it was placed at once in such intimate contact with the host tissue as was the case when the latter was injured.

On April 3, 1917, six dormant bulbs were dusted with conidia from a pure culture of strain B. XXVII, and by April 25 five of these were

strongly infected and some showed aerial mycelium arising from the lesions. On April 18, 1917, ten, clean, dormant bulbs were inoculated in the same manner with conidia from a culture of strain B. XXVII. In several days, nine of these showed numerous spots or streaks, varying in color from yellow to brown, where the conidia had been sown.

On March 30, 1917, at 5 p. m., four tulip leaves were dusted with conidia from a pure culture and the following morning at nine o'clock small water-soaked spots had appeared, a microscopical examination of the epidermis of which showed that the conidia had germinated and had penetrated the cuticle. On April 2 these spots showed a rusty color and were surrounded by translucent, water-soaked areas.

On April 1, 1917, at 4 p. m., three tulip plants in pots were inoculated with conidia of strain B. XXVII and placed under a large bell glass. On April 2, at 9 p. m., all had developed a considerable number of infections, as shown by the large number of small, yellow spots. These spots later become larger and of a somewhat reddish cast. About five days later the plants were severely diseased and showed a mycelial growth on the leaf surface. About seven days thereafter conidia were formed. On April 12 some sclerotia were noted in the leaf tissue.

On April 13, 1917, eight tulip plants were inoculated with conidia of strain B. XXVII and placed in a large moist chamber. On April 16 all showed numerous yellow-to-reddish spots on the leaves. A few similar spots appeared on the stems. By April 18, the small spots had coalesced and appeared as large, reddish lesions, some of which were covered with abundant conidiophores.

The inoculations with conidia are summarized in table 5. The plants were not mechanically injured at the time of inoculation.

TABLE 5. RESULTS FROM INOCULATING UNINJURED TULIP PLANTS WITH CONIDIA

Experiment	Culture	Plant part	Number inoculated	Number infected
16	B. XXVII	Bulb (dormant)	6	5
18	B. XXVII	Bulb (dormant)	10	9
14	B. XXVII	Leaves (detached)	4	4
15	B. XXVII	Tops	3	3
17	B. XXVII	Tops	8	8

In using mycelium as inoculum on the leaves and the stems, the lesions, after a short time, become as typical as those of the same age produced in nature. In the beginning, however, although they show the characteristic yellowing, they are not exactly like natural lesions, for they assume the shape of the agar block used in the inoculation. As the infection spreads, the region about the inoculum becomes water-soaked, then depressed, and finally dried out. At the last stage, the lesion assumes a dull gray color and produces fluffy mycelium and sometimes also a conidial layer. The lesions tend to elongate in the direction of the stem and the leaf. If an inoculation is made on the edge of a young leaf near the tip, the peculiar twisting described on page 323, under the heading *Symptoms*, results. The whole plant top may be involved as a result of such an inoculation (fig. 42). On the dormant bulbs, also, the lesions are typical (fig. 41) with a dark brown, shiny surface. In using conidia as inoculum, the lesions are typical from the start and are essentially as described on pages 319 to 328.

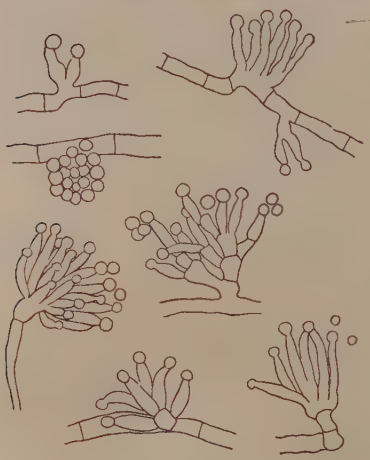

FIG. 40. MICROCONIDIA OF BOTRYTIS TULIPAE. × 600
(Camera-lucida drawing)

With but few exceptions, when infection was positive, the fungus in these experiments was re-isolated in pure culture by one of the isolation processes described under *Methods*, and checked identically with the original culture. Usually the tissue-planting method was the one employed.

In order to determine the range of parasitism of this species, a considerable number of experiments were made on both closely related and distantly related plants to find out whether *Botrytis Tulipae* is able to infect them.

The general results of these experiments appear in table 6. Certain of these, however, should be discussed more fully.

TABLE 6. RESULTS OF INOCULATION ON VARIOUS HOSTS FROM INOCULATING PLANTS NEARLY AND DISTANTLY RELATED TO THE TULIP

Plant	Inoculum	Number inoculated		Number infected	
		Injured	Uninjured	Injured	Uninjured
Lily of the valley	Mycelium	14	0	0	.....
Onion (leaves)	Mycelium	3	3	3	0
Onion (bulbs)	Mycelium	16	1	0	0
Onion (stems)	Mycelium	2	0	0	.....
Lilium sp.	Mycelium	6	6	0	0
Narcissus (leaves)	Mycelium	11	11	11	0
Narcissus (leaves)	Conidia	0	5	.....	0
Narcissus (stems)	Mycelium	3	3	3	0
Hyacinth (leaves)	Conidia	0	5	.....	0
Crocus (tops)	Mycelium	7	7	7	0
Crocus (bulbs)	Mycelium	5	5	5	1
Gladiolus	Mycelium	10	0	0	.....
Peony	Mycelium	19	0	3	.....
Potato	Mycelium	21	0	0	.....
Golden seal	Mycelium	6	0	0	.....

From table 6, the relation of *B. Tulipae* to certain plants is evident: it is not able to attack at all the lily of the valley, the lily, the gladiolus, the potato, or the goldenseal, and, probably, not the peony. However, this relation will be made clearer by a discussion of certain observations made during the attempts to cause infection on these plants.

Inoculation of onion.— Leaves and stalks of onion plants were inoculated with three different cultures, one of these a typical culture of *B. Tulipae.* In those plants that were injured at the time of inoculation, strong infection took place on the leaves but none occurred on the stems. On the uninjured plants there was no infection. To serve as checks, other plants were inoculated at the same time with

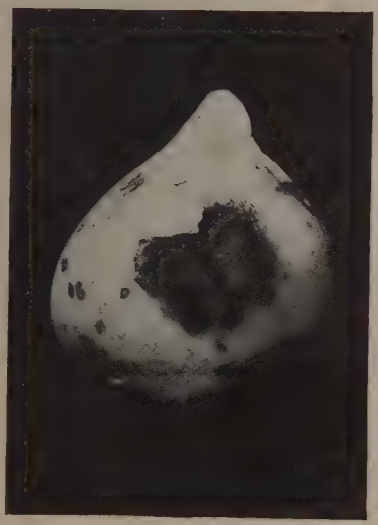

FIG. 41. RESULT OF ARTIFICIAL INOCULATION

Lesion on dormant bulb. The bulb was injured at the point of inoculation. Natural size

mycelium from a culture of the onion Botrytis, *B. Allii* Munn, and with a large sclerotial form of Botrytis from tulips — not *B. Tulipae*. From both of these inoculations a strong infection resulted on the leaves and a slight infection on the stem, in the uninjured as well as the injured plants. A similar experiment with mycelium from the same cultures was tried on onion bulbs, and here *B. Tulipae* produced no infection, while the other two species produced a strong infection, but only on the injured bulbs. It is interesting to note here that on the uninjured bulbs inoculated with *B. Tulipae*, peculiar depressions appeared in the bulb scale that at first seemed to be slight infections. However, microscopical examination showed that the mycelial threads had not penetrated. They were merely superficial. Nevertheless, beneath the mycelium some epidermal cells and other deeper-lying cells had been killed. Microscopical sections of the injured bulbs showed mycelium in the punctures made by the scalpel. It had not, however, penetrated laterally into the tissue, although some of this tissue had been killed.

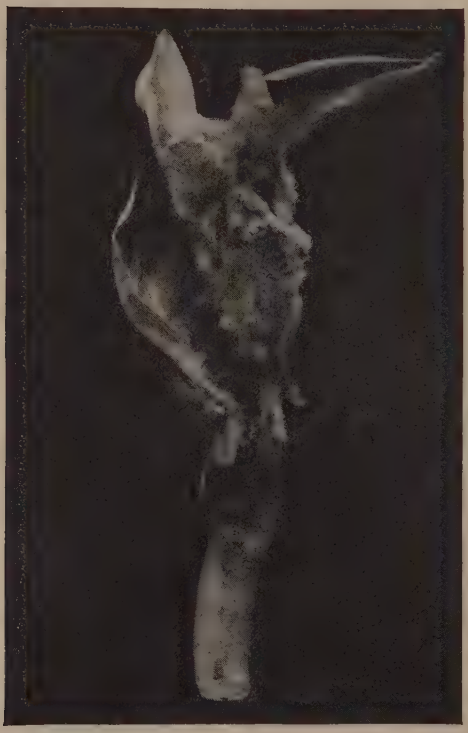

FIG. 42. RESULT OF ARTIFICIAL INOCULATION
The whole top is involved. Natural size

Inoculation of narcissus.— The result obtained on the narcissus was similar to that on the onion: strong infections appeared on the leaves and slight ones on the stems when the inoculation was performed on

mechanically injured plants. Uninjured plants were not infected. When conidia were used to inoculate narcissus, there was no sign of resultant injury, and microscopical examination of the epidermis showed the conidia to be present but not germinated.

Inoculation of hyacinth.— Detached hyacinth leaves were inoculated with conidia from pure culture. On the fifth day after inoculation, small, yellowish, depressed spots appeared. Microscopical examination of these spots showed an abundance of germinated conidia but no penetration of the epidermis by their germ tubes. Moreover, the tissue beneath the epidermis showed no mycelium.

Inoculation of crocus.— Slight infections were caused on mechanically injured crocus leaves by mycelium of *B. Tulipae*, but no infection occurred on uninjured leaves. A large sclerotial Botrytis from tulip caused no infection in either case. Very slight infections were produced on the papery scales of crocus bulbs. On injured bulbs, all the five inoculated were infected; on uninjured bulbs, only one out of five was infected. Microscopical sections of these lesions showed mycelium ramifying through the tissue, and disintegration of the cells was observed.

Discussion of parasitism

It is evident from these pathogenicity experiments that *B. Tulipae* is practically restricted to tulips. Although under certain conditions it attacks some closely related plants, even such infection occurs, almost invariably, only when there is an injury made at the point of inoculation. Furthermore, a large number of injured plants failed to become infected. When we consider the ease with which the tulip may be infected, whether mycelium or conidia be used as inoculum and whether the host plant be injured or uninjured, these apparent exceptions only make more evident its restricted parasitism. Indeed the writer believes that in those instances in which *B. Tulipae* is reported on other hosts, if the fungus were really that species, the infection took place on an injured part of the host plant. On the other hand, the pathogene shows gradation in parasitism in its feeble attempts to invade plants other than its normal host. First, there are plants such as the crocus, on which are produced only slight infections which do not spread. Next, there are plants such as the narcissus and the onion, on which the conidia do not even germinate and infection

by mycelium can begin only at an injured place. Again, there are plants such as the hyacinth, on which the conidia will germinate and cause local injury, without actually invading the plant. Finally, there is the tulip, in the case of which infections take place easily on uninjured plants.

While this series is too incomplete to be conclusive, the tendency shown is clear, and further experiments in this direction would probably furnish additional evidence of the very limited range of the parasitism of *B. Tulipae.*

Life history

Primary inoculation and infection

The fungus survives the dormant period of the bulb as mycelium or sclerotia and is planted with it in the fall. When the bulb starts into activity in the spring, the fungus starts also and sometimes spreads throughout the entire outer scale of the bulb. If the original infection is near the apex of the bulb, the shoot also is involved in the lesion and the mycelium growing from the bulb tissue infects the leaf tissue. This condition was frequently encountered in studying the disease and is well illustrated in figure 27. Usually it is only the outer, sheathing leaf that is diseased, although sometimes the whole shoot may be affected and fail to emerge from the soil. After growing in the leaf for a time, the mycelium emerges from the dead tissue and, if favorable conditions prevail, conidiophores and conidia are produced. These are formed on the aerial mycelium and also arise directly from the mycelium in the leaf. The unspecialized hyphae and the conidiophores which arise from the leaf emerge through the stomata, and in the specimens observed, only one came from each stoma.

Secondary inoculations and infections

The conidia, produced in great abundance on these first-infected leaves, furnish abundant inoculum for secondary inoculations. Although it is not improbable that they are also transported by such other agencies as insects, spattering rain, animals, and man, the conidia are for the most part scattered to the infection courts by means of the wind.

The infection courts may be any part of the tulip plant except the roots. Conidia falling on these parts germinate very quickly under proper conditions. Experiments with conidia in tulip juice and in distilled water

produced successful germination. In the former there was a good development of germ tubes overnight, while in the latter germination took place but the development of the germ tubes was poor. This experiment was conducted at room temperature. The germ tubes penetrate directly, as discussed under the heading *Pathological histology* (page 351) and cause infection. Visible evidence of infection often appears within the short period of twenty-four hours, as was demonstrated in the pathogenicity experiments. Under conditions unfavorable for germination the conidia are able to retain their viability for some time, as the following experiment illustrates.

Tulip material abundantly covered with conidia was collected on June 12, 1917, at Ithaca, New York. It was kept under laboratory conditions and the capacity of the conidia for germination was tested on June 12, June 25, July 12, and August 2. Germination of conidia was obtained at all of these dates except the last. This shows that in a dry condition the conidia retain for several weeks their ability to germinate. The lesions caused by their infections soon enlarge and produce more conidiophores and conidia, which in turn are capable of producing more infections. That these infections are continually taking place is evident from the presence of lesions of various ages on the same leaf (fig. 25).

Conidia may be carried from badly diseased tops to the bulbs, perhaps being washed down by rain. Several specimens were collected which clearly showed this. Incipient lesions were found on both the stalks and the bulbs of such plants, showing how the inoculum works down to the bulbs. These lesions increase in size and sclerotia are produced. When the bulb becomes dormant the development of the lesion is arrested and the fungus is again ready for hibernation. There is no doubt that the sclerotia retain their vitality for a long period. In fact, isolations have been made from sclerotia which have been in a resting state for several years.

Pathological histology

The material was fixed in Flemming's, in chromo-acetic, and in Gilson's fluids, was embedded in paraffin, sectioned, and stained with both Heidenhain's iron alum-haematoxylon and Flemming's triple stain. Some difficulty was experienced in sectioning lesions on the bulbs because of the numerous, large, starch grains present. In order to study the

FIG. 43. LESION ON THE OUTER BULB SCALE

Photomicrograph of cross section through lesion. The accumulation of starch in the cells, and an incipient sclerotium, are visible

penetration, certain areas on the tulip leaves were marked with india ink, inoculated by dusting with conidia, and, after various intervals, cut out, killed, fixed, and stained.

On the bulbs, typical necrotic lesions appear, which show a peculiar accumulation of starch about the diseased area. This is pictured in its general features in figure 43, and in more detail in figures 44 and 45, one of which shows a diseased area and the other a healthy one. These starch grains are heart-shaped and large. They react to iodine in potassium iodide in the usual way, and with Flemming's triple stain are colored a beautiful pink. Why they should accumulate in this manner about the lesion is not known.

The mycelium in the bulbous tissue is usually of small diameter and is both inter- and intracellular. Usually in that part of the tissue where the mycelium is advancing and the cells are not yet killed, it is intercellular, while in the older part of the lesion the hyphae penetrate into the cells as well as between them. The protoplasm of the cells at this stage is practically gone. The collapse of these empty cells causes the lesion to be depressed. Sclerotia sometimes form on the surface of the lesion. In figure 43 an incipient sclerotium may be seen.

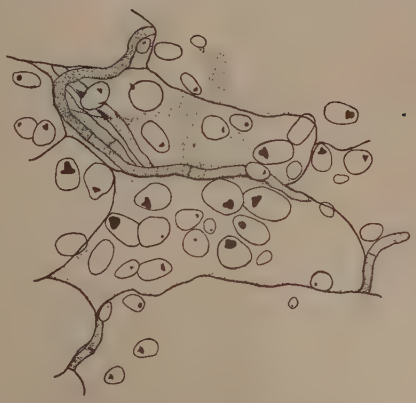

FIG. 44. STARCH ACCUMULATION IN DISEASED TISSUE. × 277

Starch cells in a diseased area of an outer bulb scale, showing numerous starch grains and intercellular mycelium. (Camera-lucida drawing)

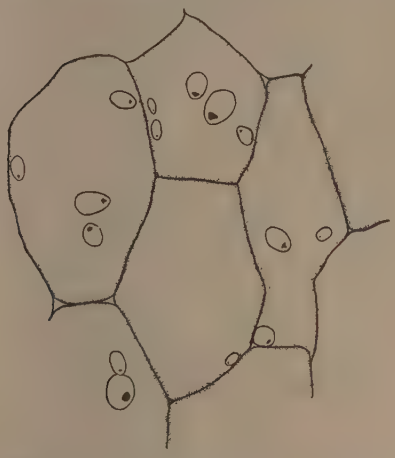

FIG. 45. HEALTHY BULB TISSUE. × 277

The amount of starch shown here may be compared with that shown in figure 44.

Vascular bundles in the bulb scale were markedly affected, and in one case the xylem had entirely disappeared while the phloem, though attacked, still remained in part. In another specimen the bundle had been disintegrated on the side toward the lesion. This involved the phloem, the cells of which stained a deeper blue. There was starch accumulation in this region.

Penetration of the fungus into the leaf tissue has been observed. No appressoria are formed by the germ tubes, which instead penetrate directly through the leaf surface, either through stomata or

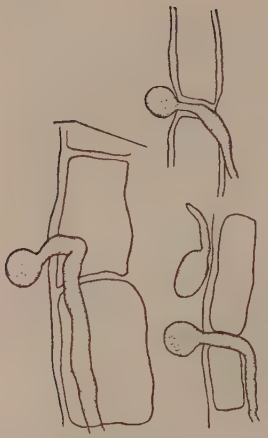

FIG. 46. PENETRATION OF LEAF TISSUE. × 277
Transverse section of epidermis. (Camera-lucida drawing)

between epidermal cells (figs. 46, 47). The germ tubes have not been observed to penetrate directly through epidermal cells. It has been noted that penetration more often occurs where the conidia are more numerous. Probably this is because of the greater enzymatic action, which hydrolyzes the cuticular substance.

The mycelium in the leaf, like that in the bulb, is both inter- and intracellular. This is shown in figures 48 and 49. After the fungus has developed for a time in the leaf tissue, a collapse of the cells results and causes the leaf to become much thinner in the diseased area. Here also, where the mycelium is still intercellular, the cells are not killed. There is injury caused in advance of the mycelium. This indicates the excretion of toxic or enzymatic substances by the pathogene.

The writer thinks that the injury caused in this disease is not due to oxalic acid. Some experiments were made to determine what the nature of the injury from oxalic acid would be. Several plants were injected hypodermically with solutions of oxalic acid of various concentrations, and lesions were produced which strikingly resembled those caused by a fungus. Furthermore, microscopical examination indicated that no fungus had been accidentally introduced. However, the concentrations were necessarily higher than those produced by fungi in culture. The work of Brown (1915) seems to show that neither oxalates nor oxalic acid take part in the toxicity of *B. cinerea*, but

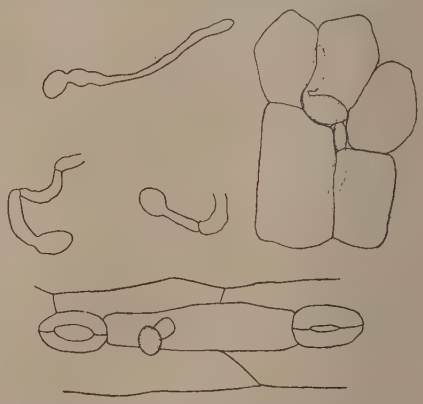

FIG. 47. PENETRATION OF LEAF TISSUE. × 277
Surface view. (Camera-lucida drawing)

FIG. 48. INTERCELLULAR MYCELIUM IN LEAF TISSUE. × 600
The cells have not yet lost their protoplasm. (Camera-lucida drawing)

that this toxicity is due to enzymatic action.

CONTROL

It has been impossible to carry control experiments far enough to justify making any definite recommendations for the control of *Botrytis Tulipae*. A consideration of the pathogene, however, makes it evident that elimination is probably of first importance. Clean bulbs, free from mycelium and sclerotia, should produce clean tulips, for it is most probable that in these forms the pathogene is carried on the bulbs. Although the disease may possibly be attributed to infested soil, it often occurs on tulips grown in soil in which heretofore no tulips have been grown.

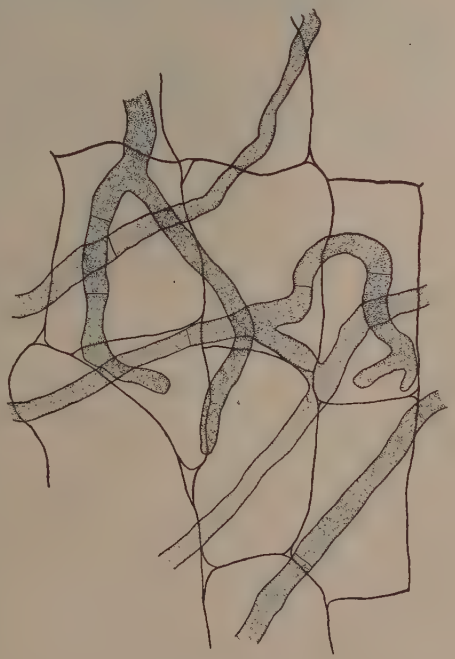

FIG. 49. INTRACELLULAR MYCELIUM IN LEAF TISSUE. × 600
The cells of the leaf are devoid of contents. (Camera-lucida drawing)

FIG. 50. PROLIFERATION OF CONIDIOPHORE. × 840
Showing new branches arising from conidial cluster. (Photomicrograph)

Until further experiments have been made, soil treatment cannot be recommended. Carbolineum has been recommended in the literature as a disinfectant for soil (Elenkin, 1911). Klebahn (1904:33) criticizes this method unfavorably, saying that not even weeds will grow in soil thus treated.

Experiments made at Madison, Wisconsin, in 1917, showed that spraying tulips with bordeaux mixture 5–5–50 caused considerable injury to both the leaves and the flowers, besides giving them an unsightly appearance. Accordingly this treatment is not to be recommended.

From the present knowledge of this disease the following measures seem advisable:

1. Selection of clean bulbs, free from lesions and sclerotia. When the sclerotia occur only on the outer papery scale this should be removed and burned. It is well also to inspect the old stalk of the previous year, if this still remains attached, for it frequently bears sclerotia (fig. 22).

2. Careful handling, to avoid injuring the bulbs, as infection takes place more readily on injured bulbs than on healthy ones.

3. Storage of the bulbs under proper conditions of temperature and humidity. The temperature should be kept as low as possible without injury to the bulbs, preferably about 40° F. The humidity also should be low. These conditions are especially desirable, as they retard the development of any small lesions that may be present on the bulbs at the time of storage, and prevent the germination of any conidia that may be on their surface.

4. Removal and destruction of diseased plants when they appear in the field or the beds. This will limit, if it does not entirely prevent, secondary infections.

SUMMARY

An investigation of the tulip disease caused by *Botrytis Tulipae* (Libert) comb. nov. shows that it is present throughout the United States and that it was probably introduced with the introduction of tulip bulbs. Reports of the disease show that it has been in this country at least since 1901.

Under normal conditions this disease is restricted to the genus Tulipa and within this genus practically all varieties are susceptible. One instance of apparent immunity is the variety Baronne de la Tonnaye, which, during an epidemic of Botrytis blight, showed no evidence of the disease.

Counts made in the spring of 1917, at Ithaca, on one variety of late tulips, Spathulata, showed 100 per cent of the leaves and 98 per cent of the stalks to be affected. Other varieties were similarly infected. These infections were traced to the bulbs, of which the variety Spathulata showed 4.6 per cent with unmistakable Botrytis lesions and the Mrs. Grover Cleveland variety, 5.2 per cent.

The disease is easily recognized on the bulbs when the fungous sclerotia are present in the lesions. On the leaves, the flower stalks, and the flowers, a severe blighting frequently takes place.

Studies of the literature and herbarium specimens show that the disease under consideration is to be ascribed to *Botrytis Tulipae* (Libert) comb. nov.[4]

Cultural studies have demonstrated that both the small sclerotia on the bulbs and the conidial form on the leaves and other parts of the tulip plant are stages of one and the same fungus, namely, *Botrytis Tulipae*.

[4] In recent literature the fungus has gone by the name *Botrytis parasitica* Cavara, but the specific name of Libert has priority.

The morphology of the parasite has been investigated in some detail. The manner of formation and attachment of the conidia has been brought out, microconidia have been demonstrated for this species and conidial measurements show that while the variation in size is from 12–24 x 10–20, the greater number of spores measure 16–17 x 9–10.

Conidial production, which rarely takes place in pure cultures under ordinary conditions, was found to occur abundantly when plate cultures were partially dried. Abundant conidia were also formed on sterilized tulip leaves in large test tubes.

The parasitism of *B. Tulipae* has been fully demonstrated by numerous infections brought about by the use of pure cultures of the organism. Inoculations of other plants, both nearly and distantly related, while showing the parasite to be restricted to tulips, show also that the parasite exhibits a weak and varying degree of ability to attack other plants.

Hibernation is by means of sclerotia which live over the winter on the bulbs. Infection spreads from these bulbs to the developing shoots, where abundant conidia are produced. These primary lesions serve as the source of inoculum for secondary infection. The conidia produced in this manner retain their vitality for several weeks.

Sections through lesions on the bulb show an accumulation of starch about the diseased area. In the penetration of the tissue by conidial germ tubes, no appressoria are formed and the germ tubes penetrate directly through the epidermis or through the stomata.

Although extensive control experiments have not been made, it is recommended that clean bulbs, careful handling of bulbs, proper storage, and systematic removal and destruction of diseased plants in the field will largely hold the disease in check.

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